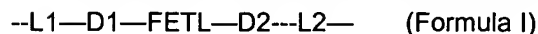


**Listing of Claims:**

1. (Currently Amended) A fluorescence energy transfer dye comprising ~~having the~~ Formula I:



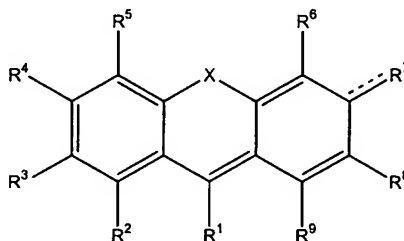
where:

L1 is a link that is a chemical bond for attachment to a probe or target, for attachment to a solid support, or is absent;

L2 is a link that is a chemical bond for attachment to a probe or target, for attachment to a solid support, or is absent;

FETL is a fluorescence energy transfer linker ~~comprising that is~~ a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;

D1 is a donor dye represented by the formula:



D1

where:

X is O or C(R\*R\*\*), where R\* and R\*\* are independently lower alkyl or -CH<sub>2</sub>-Z;

R<sup>1</sup> is H, CF<sub>3</sub>, perfluoropropyl, lower alkyl acid, substituted aryl, substituted heteroaryl or Z;

R<sup>2</sup> is H, halo, SO<sub>3</sub><sup>-</sup>, or is taken together with R<sup>3</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>3</sup> is halo, Z, or is taken together with R<sup>2</sup> and/or R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>4</sup> is =O or OH, -N(R<sup>4'</sup>R<sup>4''</sup>) or =N<sup>+</sup>(R<sup>4'</sup>R<sup>4''</sup>), or is taken together with R<sup>3</sup> and/or R<sup>5</sup> to form an optionally substituted fused ring having 5 to 7 atoms,

where R<sup>4'</sup> is H, lower alkyl or L1, and

R<sup>4''</sup> is H, lower alkyl or CH<sub>2</sub>-Z;

R<sup>5</sup> is H, halo, Z, or is taken together with R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>6</sup> is H, halo, Z, or is taken together with R<sup>7</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

$R^7$  is  $=O$  or  $OH$ ,  $-N(R^7R^7)$  or  $=N^+(R^7R^7)$ , or is taken together with  $R^6$  and/or  $R^8$  to form an optionally substituted fused ring having 5 to 7 atoms,

where  $R^7$  is H, lower alkyl or L1, and

$R^7$  is H, lower alkyl or  $CH_2-Z$ ;

$R^8$  is halo, Z, or is taken together with  $R^7$  and/or  $R^9$  to form an optionally substituted fused ring having 5 to 7 atoms;

$R^9$  is H, halo,  $SO_3^-$ , or is taken together with  $R^8$  to form an optionally substituted fused ring having 5 to 7 atoms; and

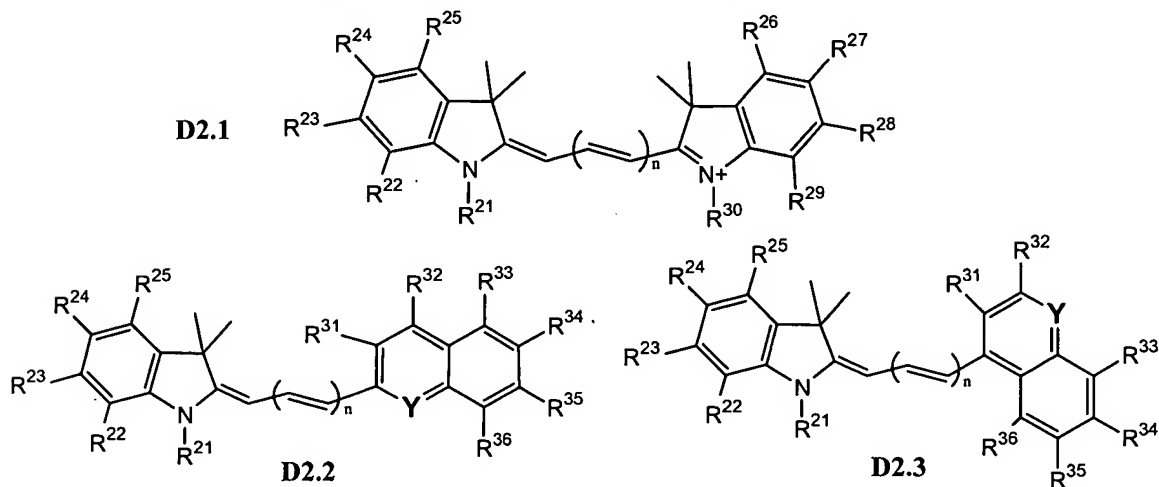
Z is a group of the formula:  $-Z^*-Z^1-$  (linkage to L1, L2 or FETL), where:

$Z^*$  is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl;

$Z^1$  is  $-C(O)-$ ,  $-N(Z^2)-$ ,  $-CH_2-O-$ ,  $-CH_2-C(O)-$ ,  $-CH_2-N(Z^2)-$ ,  $-CH_2-S-$ ,  $-CH_2-S(O)-$ ,  $-CH_2-S(O_2)-$  or is absent; and

$Z^2$  is H,  $C_1$  to  $C_8$  optionally substituted lower alkyl, or optionally substituted aryl; and

D2 is an acceptor/reporter dye represented by formula D1 or by a formula of the group:



where:

at least one of  $R^{21}$  to  $R^{36}$  is joined to FETL,

$n$  is zero, 1, 2 or 3;

$R^{21}$  and  $R^{30}$  are independently  $-CH_2-Z$ , activated lower alkyl, or optionally substituted aryl/alkyl;

$R^{22}$  to  $R^{29}$  are independently H,  $SO_3^-$ , or optionally substituted alkyl, or  $R^{22}$  and  $R^{23}$ ,  $R^{23}$  and  $R^{24}$ ,  $R^{24}$  and  $R^{25}$ ,  $R^{26}$  and  $R^{27}$ ,  $R^{27}$  and  $R^{28}$ , and/or  $R^{28}$  and  $R^{29}$  taken together form an optionally substituted fused ring having 6 atoms;

$R^{31}$  and  $R^{32}$  are independently H, optionally substituted alkyl, aryl, or taken together form an optionally substituted fused ring having 6 atoms;

$R^{33}$  to  $R^{36}$  are independently H,  $SO_3^-$ , optionally substituted alkyl, aryl, or  $R^{33}$  and  $R^{34}$ ,  $R^{34}$  and  $R^{35}$ , and/or  $R^{35}$  and  $R^{36}$  taken together form an optionally substituted fused ring having 6 atoms; and

Y is  $-O-$  or  $-N(Y^1)-$  where  $Y^1$  is  $-CH_2-Z$ , activated lower alkyl, or optionally substituted alkylaralkyl;

provided that at least one of  $R^3$  to  $R^8$  is Z where Z\* is optionally substituted aryl or optionally substituted heteroaryl, ~~or a probe, target, and/or support conjugate thereof.~~

2. (Currently Amended) The fluorescence energy transfer dye of Claim 1 having one or more of the following:

$R^1$  is H,  $CF_3$ , perfluoropropyl, lower alkyl acid, 5-6 membered mono or 10-12 membered fused substituted aryl or ~~heteroaryl~~heteroaryl, or Z;

$R^2$  is H, halo,  $SO_3^-$ , or is taken together with  $R^3$  to form an optionally substituted fused 6-membered aryl ring;

$R^3$  is halo, Z, or is taken together with  $R^2$  and/or  $R^4$  to form an optionally substituted fused 6-membered ring;

$R^4$  is  $=O$  or  $OH$ ,  $-N(R^4'R^4'')$  or  $=N^+(R^4'R^4'')$ , or is taken together with  $R^3$  and/or  $R^5$  to form an optionally substituted fused 6-membered ring;

$R^5$  is H, halo, Z, or is taken together with  $R^4$  to form an optionally substituted fused 6-membered ring;

$R^6$  is H, halo, Z, or is taken together with  $R^7$  to form an optionally substituted fused 6-membered ring;

$R^7$  is  $=O$  or  $OH$ ,  $-N(R^7'R^7'')$  or  $=N^+(R^7'R^7'')$ , or is taken together with  $R^6$  and/or  $R^8$  to form an optionally substituted fused 6-membered ring;

$R^8$  is halo, Z, or is taken together with  $R^7$  and/or  $R^9$  to form an optionally substituted fused 6-membered ring;

$R^9$  is H, halo,  $SO_3^-$ , or is taken together with  $R^8$  to form an optionally substituted fused 6-membered aryl ring;

$R^4$  is  $-N(R^4'R^4'')$  or  $=N^+(R^4'R^4'')$  and  $R^7$  is  $-N(R^7'R^7'')$  or  $=N^+(R^7'R^7'')$  when X is  $C(R^*R^{**})$ ;

Z is a group of the formula:  $-Z^*-Z^1-$  (linkage to L1, L2 or FETL), where:

$Z^*$  is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl,

$Z^1$  is  $-C(O)-$ ,  $-N(Z^2)-$ ,  $-CH_2-O-$ ,  $-CH_2-C(O)-$ ,  $-CH_2-N(Z^2)-$ ,  $-CH_2-S-$ ,  $-CH_2-S(O)-$ ,  $-CH_2-S(O_2)-$  or is absent, and

$Z^2$  is H, or is  $C_1$  to  $C_8$  lower alkyl or aryl optionally substituted with  $SO_3^-$ ,  $COOH$ ,  $NH_2$ ,  $CH_2NH_2$ ,  $SH$ , or  $SCH_3$ ;

~~FETL comprises a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;~~  
 $R^{21}$  or  $R^{30}$  is carboxy-naphthyl-methyl, mono- or di-*ortho*-substituted benzyl having an *ortho*-, *meta*- or *para*- carbonyl or activating group, or an activated  $C_1$  to  $C_6$  lower alkyl;  
 $R^{22}$  to  $R^{29}$  are independently H,  $SO_3^-$  or optionally substituted alkyl, or  $R^{22}$  and  $R^{23}$ ,  $R^{23}$  and  $R^{24}$ ,  $R^{24}$  and  $R^{25}$ ,  $R^{26}$  and  $R^{27}$ ,  $R^{27}$  and  $R^{28}$ , and/or  $R^{28}$  and  $R^{29}$  taken together form a fused, sulfonated 6-membered aryl ring;  
 $R^{31}$  and  $R^{32}$  are independently H, optionally substituted alkyl, aryl, or taken together form a fused, sulfonated 6-membered carbocyclic or heterocyclic ring;  
 $R^{33}$  to  $R^{36}$  are independently H,  $SO_3^-$ , optionally substituted alkyl, aryl, or  $R^{33}$  and  $R^{34}$ ,  $R^{34}$  and  $R^{35}$ , and/or  $R^{35}$  and  $R^{36}$  taken together form a fused, sulfonated 6-membered carbocyclic or heterocyclic ring; and/or  
at least one of  $R^{22}$  to  $R^{29}$  or  $R^{33}$  to  $R^{36}$  is  $SO_3^-$ .

3. (Currently Amended) The fluorescence energy transfer dye of Claim 2 having one or more of the following:

$R^1$  is H,  $CF_3$ , perfluoropropyl, lower alkyl acid, an optionally substituted *ortho*-benzoic acid, or Z;

$R^2$  is H, halo or  $SO_3^-$ ;

$R^3$  is halo or Z;

$R^4$  is =O or OH,  $-N(R^4'R^4'')$  or  $=N^+(R^4'R^4'')$ ;

$R^5$  is H, halo or Z;

$R^6$  is H, halo or Z;

$R^7$  is =O or OH,  $-N(R^7'R^7'')$  or  $=N^+(R^7'R^7'')$ ;

$R^8$  is Z;

$R^9$  is H, halo or  $SO_3^-$ ;

Z is a group of the formula:  $-Z^*-Z^1-$  (linkage to L1, L2 or FETL), where:

$Z^*$  is an optionally substituted aryl or heteroaryl of the group: phenyl, adamantyl, norboranyl, biphenyl, naphthyl, furanyl, bifuranyl, thiophenyl, bithienyl, pyrrolyl, indolyl, isoindolyl, benzofuranyl, isobenzofuranyl, benzothiophenyl, benzothienyl, chromenyl and isochromenyl, and

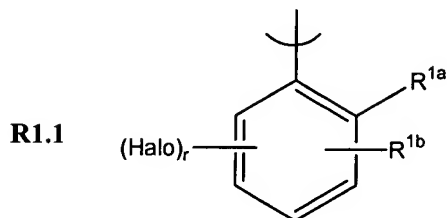
$Z^1$  is  $-C(O)-$ ,  $-N(H)-$ ,  $-CH_2-O-$ ,  $-CH_2-C(O)-$ ,  $-CH_2-N(H)-$ , or is absent;

$R^{22}$  to  $R^{29}$  are independently H,  $SO_3^-$  or optionally substituted alkyl, or  $R^{24}$  and  $R^{25}$  and/or  $R^{26}$  and  $R^{27}$  taken together form a fused, sulfonated 6-membered aryl ring;

$R^{31}$  and  $R^{32}$  are independently H, optionally substituted alkyl or aryl; and/or

$R^{33}$  to  $R^{36}$  are independently H,  $SO_3^-$ , optionally substituted alkyl or aryl.

4. (Previously Amended) The fluorescence energy transfer dye of Claim 3 where R<sup>1</sup> is a group represented by Formula R1.1:



where:

R<sup>1a</sup> is H, halo or -C(O)O<sup>-</sup>;

R<sup>1b</sup> is H, halo or -C(O)-(linkage to L1, L2 or FETL);

R<sup>1c</sup> is halo; and

r is 0, 1, 2 or 3.

5. (Currently Amended) The fluorescence energy transfer dye of Claim 1 where at least one of R<sup>3</sup> to R<sup>8</sup> is Z where Z\* is optionally substituted aryl selected from phenyl, ~~adamantyl~~adamantyl, norboranyl, biphenyl and naphthyl.
6. (Original) The fluorescence energy transfer dye of Claim 5 where Z\* is phenyl and Z<sup>1</sup> is -C(O)-.
7. (Currently Amended) The fluorescence energy transfer dye of Claim 1 where:
- R<sup>1</sup> is H, CF<sub>3</sub>, perfluoropropyl, lower alkyl acid, an optionally substituted *ortho*-benzoic acid, or Z;
  - R<sup>2</sup> is H, halo or SO<sub>3</sub><sup>-</sup>;
  - R<sup>3</sup> is halo or Z;
  - R<sup>4</sup> is =O or OH, -N(R<sup>4'</sup>R<sup>4''</sup>) or =N<sup>+</sup>(R<sup>4'</sup>R<sup>4''</sup>);
  - R<sup>5</sup> is H, halo or Z;
  - R<sup>6</sup> is H, halo or Z;
  - R<sup>7</sup> is =O or OH, -N(R<sup>7'</sup>R<sup>7''</sup>) or =N<sup>+</sup>(R<sup>7'</sup>R<sup>7''</sup>);
  - R<sup>8</sup> is Z;
  - R<sup>9</sup> is H, halo or SO<sub>3</sub><sup>-</sup>;
- Z is a group of the formula: -Z\*-Z<sup>1</sup>- (linkage to L1, L2 or FETL), where:
- Z\* is an optionally substituted aryl or heteroaryl of the group: phenyl, ~~adamantyl~~adamantyl, norboranyl, biphenyl, naphthyl, furanyl, bifuranyl, thiophenyl, bithienyl, pyrrolyl, indolyl, isoindolyl, benzofuranyl,

isobenzofuranyl, benzothiophenyl, benzothienyl, chromenyl and isochromenyl, and

$Z^1$  is  $-C(O)-$ ,  $-N(H)-$ ,  $-CH_2-O-$ ,  $-CH_2-C(O)-$ ,  $-CH_2-N(H)-$ , or is absent;

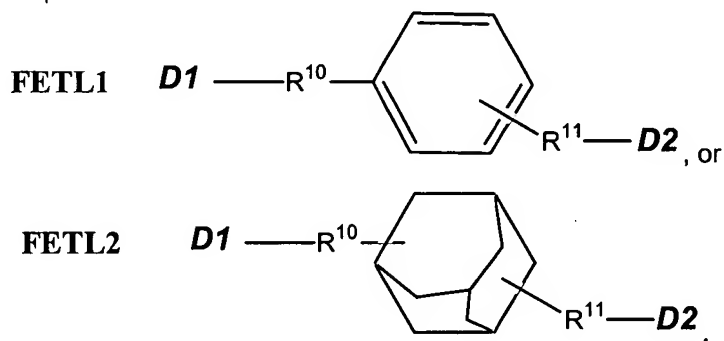
$R^{21}$  or  $R^{30}$  is carboxy-naphthyl-methyl, mono- or di-*ortho*-substituted benzyl having an *ortho*-, *meta*- or *para*- carbonyl or activating group, or an activated  $C_1$  to  $C_6$  lower alkyl;

$R^{22}$  to  $R^{29}$  are independently H,  $SO_3^-$  or optionally substituted alkyl, or  $R^{24}$  and  $R^{25}$  and/or  $R^{26}$  and  $R^{27}$  taken together form a fused, sulfonated 6-membered aryl ring;

$R^{31}$  and  $R^{32}$  are independently H, optionally substituted alkyl or aryl; and

$R^{33}$  to  $R^{36}$  are independently H,  $SO_3^-$ , optionally substituted alkyl or aryl.

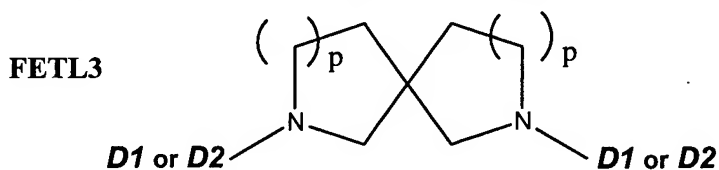
8. (Previously Amended) The fluorescence energy transfer dye of Claim 1 where FETL is represented by a formula of the group:



where:

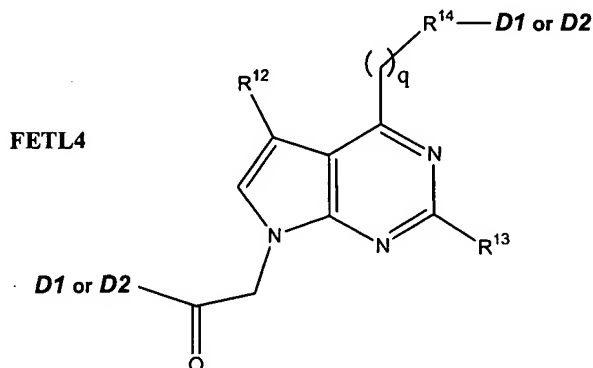
$R^{10}$  is  $-C(O)-$ ,  $-N(H)-CH_2-$ ,  $-S-C(O)-$ ,  $-O-C(S)-$  or  $-S-CH_2-$ ; and

$R^{11}$  is  $-C(O)-$ ,  $-CH_2-N(H)-$ ;  $-C(O)-S-$ ,  $-C(S)-O-$  or  $CH_2-S-$ ;



where:

p is independently 0, 1, 2 or 3; and



where:

q is zero or 1;

R<sup>12</sup> is H, halo, or optionally substituted-alkyl, -alkenyl, -alkynyl or -aryl;

R<sup>13</sup> is H, SO<sub>3</sub><sup>-</sup> or optionally substituted-alkyl, -alkenyl, -alkynyl or -aryl; and

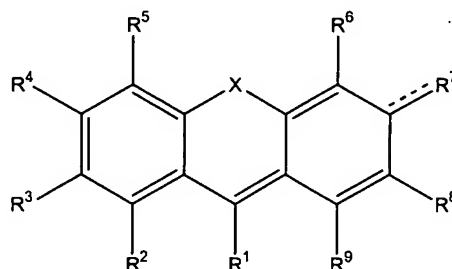
R<sup>14</sup> is a secondary or tertiary amine or heterocyclyl, particularly N(H) or piperazine.

9. (Currently Amended) The fluorescence energy transfer dye of Claim 8 where FETL is FETL1 and:

R<sup>10</sup> is -C(O)- or -N(H)-CH<sub>2</sub>-; and

R<sup>11</sup> is -C(O)- or -CH<sub>2</sub>-N(H)-.

10. (Currently Amended) A compound represented by the formula:



D1

where:

X is O or C(R\*R\*\*), where R\* and R\*\* are independently lower alkyl or -CH<sub>2</sub>-Z;

R<sup>1</sup> is H, CF<sub>3</sub>, perfluoropropyl, lower alkyl acid, substituted aryl, substituted heteroaryl or Z;

R<sup>2</sup> is H, halo, SO<sub>3</sub><sup>-</sup>, or is taken together with R<sup>3</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>3</sup> is halo, Z, or is taken together with R<sup>2</sup> and/or R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>4</sup> is =O or OH, -N(R<sup>4'</sup>R<sup>4''</sup>) or =N<sup>+</sup>(R<sup>4'</sup>R<sup>4''</sup>), or is taken together with R<sup>3</sup> and/or R<sup>5</sup> to form an optionally substituted fused ring having 5 to 7 atoms,

where R<sup>4'</sup> is H, lower alkyl or L1, and

R<sup>4''</sup> is H, lower alkyl or CH<sub>2</sub>-Z;

R<sup>5</sup> is H, halo, Z, or is taken together with R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>6</sup> is H, halo, Z, or is taken together with R<sup>7</sup> to form an optionally substituted fused ring having 5 to 7 atoms;

R<sup>7</sup> is =O or OH, -N(R<sup>7'</sup>R<sup>7''</sup>) or =N<sup>+</sup>(R<sup>7'</sup>R<sup>7''</sup>), or is taken together with R<sup>6</sup> and/or R<sup>8</sup> to form an optionally substituted fused ring having 5 to 7 atoms,

where  $R^7$  is H, lower alkyl or L1, and

$R^7$  is H, lower alkyl or  $CH_2-Z$ ;

$R^8$  is halo, Z, or is taken together with  $R^7$  and/or  $R^9$  to form an optionally substituted fused ring having 5 to 7 atoms;

$R^9$  is H, halo,  $SO_3^-$ , or is taken together with  $R^8$  to form an optionally substituted fused ring having 5 to 7 atoms; and

Z is a group of the formula:  $-Z^*-Z^1-$  (linkage to L1, L2 or FETL), where:

$Z^*$  is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl;

$Z^1$  is  $-C(O)-$ ,  $-N(Z^2)-$ ,  $-CH_2-O-$ ,  $-CH_2-C(O)-$ ,  $-CH_2-N(Z^2)-$ ,  $-CH_2-S-$ ,  $-CH_2-S(O)-$ ,  $-CH_2-S(O_2)-$  or is absent; and

$Z^2$  is H,  $C_1$  to  $C_8$  optionally substituted lower alkyl, or optionally substituted aryl;

L1 being a link that is a chemical bond for attachment to a probe or target, for attachment to a solid support, or is absent;

L2 being a link that is a chemical bond for attachment to a probe or target, for attachment to a solid support, or is absent; and

FETL being a fluorescence energy transfer linker ~~comprising that is~~ a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;

provided that at least one of  $R^3$  to  $R^8$  is Z, where  $Z^*$  is optionally substituted aryl or optionally substituted heteroaryl, ~~or a FETL, probe, target and/or support-conjugate thereof.~~

11. (Currently Amended) The compound of Claim 10 having one or more of the following:

$R^1$  is H,  $CF_3$ , perfluoropropyl, lower alkyl acid, 5-6 membered mono or 10-12 membered fused substituted aryl or ~~heteroaryl~~ heteroaryl, or Z;

$R^2$  is H, halo,  $SO_3^-$ , or is taken together with  $R^3$  to form an optionally substituted fused 6-membered aryl ring;

$R^3$  is halo, Z, or is taken together with  $R^2$  and/or  $R^4$  to form an optionally substituted fused 6-membered ring;

$R^4$  is  $=O$  or  $OH$ ,  $-N(R^4'R^4'')$  or  $=N^+(R^4'R^4'')$ , or is taken together with  $R^3$  and/or  $R^5$  to form an optionally substituted fused 6-membered ring;

$R^5$  is H, halo, Z, or is taken together with  $R^4$  to form an optionally substituted fused 6-membered ring;

$R^6$  is H, halo, Z, or is taken together with  $R^7$  to form an optionally substituted fused 6-membered ring;



$R^7$  is  $=O$  or  $OH$ ,  $-N(R^7R^7)$  or  $=N^+(R^7R^7)$ , or is taken together with  $R^6$  and/or  $R^8$  to form an optionally substituted fused 6-membered ring;

$R^8$  is halo,  $Z$ , or is taken together with  $R^7$  and/or  $R^9$  to form an optionally substituted fused 6-membered ring;

$R^9$  is  $H$ , halo,  $SO_3^-$ , or is taken together with  $R^8$  to form an optionally substituted fused 6-membered aryl ring;

$R^4$  is  $-N(R^4R^4)$  or  $=N^+(R^4R^4)$  and  $R^7$  is  $-N(R^7R^7)$  or  $=N^+(R^7R^7)$  when  $X$  is  $C(R^*R^{**})$ ; and/or  $Z$  is a group of the formula:  $-Z^*-Z^1-$  (linkage to **L1**, **L2** or **FETL**), where:

$Z^*$  is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl,

$Z^1$  is  $-C(O)-$ ,  $-N(Z^2)-$ ,  $-CH_2-O-$ ,  $-CH_2-C(O)-$ ,  $-CH_2-N(Z^2)-$ ,  $-CH_2-S-$ ,  $-CH_2-S(O)-$ ,  $-CH_2-S(O_2)-$  or is absent, and

$Z^2$  is  $H$ , or is  $C_1$  to  $C_8$  lower alkyl or aryl optionally substituted with  $SO_3^-$ ,  $COOH$ ,  $NH_2$ ,  $CH_2NH_2$ ,  $SH$ , or  $SCH_3$ .

12. (Currently Amended) A fluorescence energy transfer dye conjugate represented by Formula II:

Probe--L1—(D1 or D2)—FETL—(low affinity false target) (Formula II)

where:

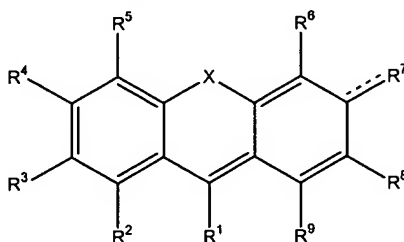
Probe is a polynucleotide, antibody, triglyceride, low density lipoprotein or lectin;

L1 is a link that is a chemical bond for attachment to the Probe;

FETL is a fluorescence energy transfer linker comprising that is a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;

low affinity false target is an analyte for the Probe disposed on FETL to deactivate, block or otherwise prevent coupling of FETL to a corresponding D2 or D1 until said low affinity false target is displaced by a higher affinity true target;

D1 is a donor dye represented by the formula:



D1

where:

X is O or C(R<sup>\*</sup>R<sup>\*\*</sup>), where R<sup>\*</sup> and R<sup>\*\*</sup> are independently lower alkyl or -CH<sub>2</sub>-Z;  
R<sup>1</sup> is H, CF<sub>3</sub>, perfluoropropyl, lower alkyl acid, substituted aryl, substituted heteroaryl or Z;  
R<sup>2</sup> is H, halo, SO<sub>3</sub><sup>-</sup>, or is taken together with R<sup>3</sup> to form an optionally substituted fused ring having 5 to 7 atoms;  
R<sup>3</sup> is halo, Z, or is taken together with R<sup>2</sup> and/or R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;  
R<sup>4</sup> is =O or OH, -N(R<sup>4'</sup>R<sup>4''</sup>) or =N<sup>+</sup>(R<sup>4'</sup>R<sup>4''</sup>), or is taken together with R<sup>3</sup> and/or R<sup>5</sup> to form an optionally substituted fused ring having 5 to 7 atoms,  
where R<sup>4'</sup> is H, lower alkyl or L1, and  
R<sup>4''</sup> is H, lower alkyl or CH<sub>2</sub>-Z;  
R<sup>5</sup> is H, halo, Z, or is taken together with R<sup>4</sup> to form an optionally substituted fused ring having 5 to 7 atoms;  
R<sup>6</sup> is H, halo, Z, or is taken together with R<sup>7</sup> to form an optionally substituted fused ring having 5 to 7 atoms;  
R<sup>7</sup> is =O or OH, -N(R<sup>7'</sup>R<sup>7''</sup>) or =N<sup>+</sup>(R<sup>7'</sup>R<sup>7''</sup>), or is taken together with R<sup>6</sup> and/or R<sup>8</sup> to form an optionally substituted fused ring having 5 to 7 atoms,  
where R<sup>7'</sup> is H, lower alkyl or L1, and  
R<sup>7''</sup> is H, lower alkyl or CH<sub>2</sub>-Z;  
R<sup>8</sup> is halo, Z, or is taken together with R<sup>7</sup> and/or R<sup>9</sup> to form an optionally substituted fused ring having 5 to 7 atoms;  
R<sup>9</sup> is H, halo, SO<sub>3</sub><sup>-</sup>, or is taken together with R<sup>8</sup> to form an optionally substituted fused ring having 5 to 7 atoms; and

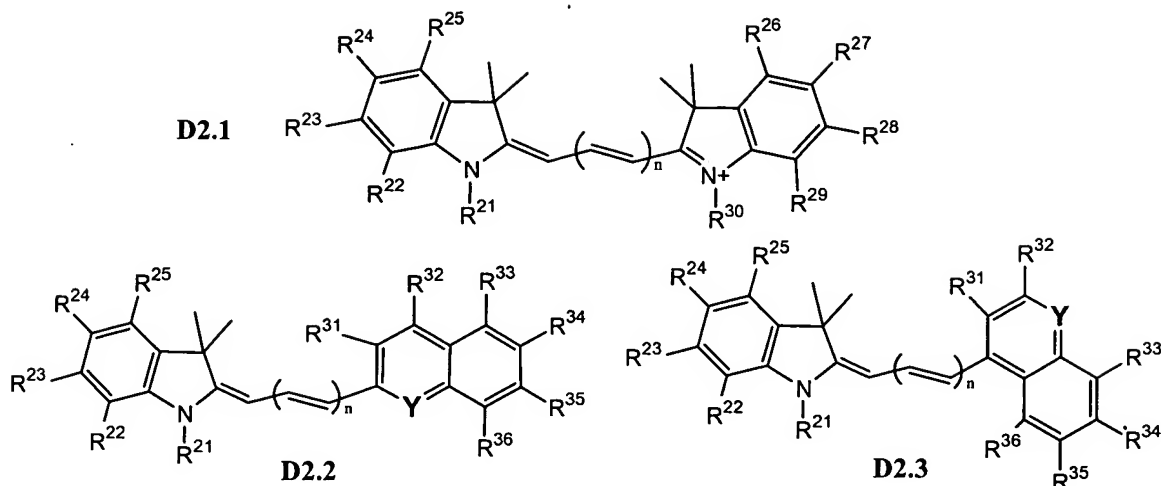
Z is a group of the formula: -Z<sup>\*</sup>-Z<sup>1</sup>- (**linkage to L1, L2 or FETL**), where:

Z<sup>\*</sup> is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl;

Z<sup>1</sup> is -C(O)-, -N(Z<sup>2</sup>)-, -CH<sub>2</sub>-O-, -CH<sub>2</sub>-C(O)-, -CH<sub>2</sub>-N(Z<sup>2</sup>)-, -CH<sub>2</sub>-S-, -CH<sub>2</sub>-S(O)-, -CH<sub>2</sub>-S(O<sub>2</sub>)- or is absent; and

Z<sup>2</sup> is H, C<sub>1</sub> to C<sub>8</sub> optionally substituted lower alkyl, or optionally substituted aryl; and

D2 is an acceptor/reporter dye represented by formula D1 or by a formula of the group:



where:

at least one of  $R^{21}$  to  $R^{36}$  is joined to FETL,

$n$  is zero, 1, 2 or 3;

$R^{21}$  and  $R^{30}$  are independently  $-\text{CH}_2\text{-Z}$ , activated lower alkyl, or optionally substituted aralkyl or aralkyl;

$R^{22}$  to  $R^{29}$  are independently H,  $\text{SO}_3^-$ , or optionally substituted alkyl, or  $R^{22}$  and  $R^{23}$ ,  $R^{23}$  and  $R^{24}$ ,  $R^{24}$  and  $R^{25}$ ,  $R^{26}$  and  $R^{27}$ ,  $R^{27}$  and  $R^{28}$ , and/or  $R^{28}$  and  $R^{29}$  taken together form an optionally substituted fused ring having 6 atoms; and

$R^{31}$  and  $R^{32}$  are independently H, optionally substituted alkyl, aryl, or taken together form an optionally substituted fused ring having 6 atoms;

$R^{33}$  to  $R^{36}$  are independently H,  $\text{SO}_3^-$ , optionally substituted alkyl, aryl, or  $R^{33}$  and  $R^{34}$ ,  $R^{34}$  and  $R^{35}$ , and/or  $R^{35}$  and  $R^{36}$  taken together form an optionally substituted fused ring having 6 atoms; and

$Y$  is  $-\text{O}-$  or  $-\text{N}(\text{Y}^1)-$  where  $\text{Y}^1$  is  $-\text{CH}_2\text{-Z}$ , activated lower alkyl, or optionally substituted aralkyl.

13. (Currently Amended) A fluorescence energy transfer assay for determining the presence of a target site in a substance requiring no pre-analysis purification for removal of unbound dye, comprising the steps of:

- (a) contacting a substance to be tested and a target site specific Probe-L1-(D1 or D2)-FETL-(low affinity false target) conjugate of Claim 12 in a suitable assay vessel under conditions suitable for preferential binding of the Probe to the target site, as opposed to the low affinity false target;
- (b) introducing into the vessel a second D2 or D1 fluorescence energy transfer dye having an activated site for coupling to the FETL ~~into the vessel~~, under conditions suitable for

coupling to FETL where the low affinity false target is not bound to the Probe,  
wherein the second dye is the other of the D1 or D2 found in the conjugate;

(c) causing D1 to absorb energy; and

(d) measuring the level of D2 emission, wherein emission from D2 upon absorption of energy  
by D1 indicates the presence of the target site in the substance.

14-16. (Cancelled)

17. (Currently Amended) A proximity assay comprising the steps of :

(a) contacting a substance to be tested and a target-site-specific donor dye in a suitable  
assay vessel;

(b) introducing a target-site-specific fluorescence energy transfer reporter dye conjugate of  
Claim 4-19 into the vessel, where said reporter dye's target is either spatially  
proximate to said donor dye target or specific for a given target to be tested for  
spatial proximity to said donor dye target, and said reporter dye's energy absorption  
spectra overlaps the emission spectra of said donor dye;

(c) causing the donor dye to absorb energy; and

(d) measuring the level of reporter dye emission, wherein emission from the reporter dye  
upon absorption of energy by the donor dye indicates the proximity of the donor dye  
target and the reporter dye target in the substance.

18. (Currently Amended) The proximity assay of Claim 17 wherein:

step (b) further comprises introducing two or more target-site-specific fluorescence energy  
transfer reporter dye conjugates of Claim 4-19 into the vessel, said reporter dyes  
having energy absorption spectra overlapping the emission spectra of said donor  
dye, distinct emission spectra, and having different targets to be tested for spatial  
proximity to said donor dye target; and

step (d) further comprises measuring the level of reporter dye emission at the wavelengths  
characteristic of said reporter dyes, wherein emission characteristic of each reporter  
dye upon absorption of energy by the donor dye indicates the respective proximity of  
the donor dye target to each corresponding reporter dye target in the substance.

19. (New) A conjugate comprising the dye of claim 1 conjugated to a probe, a target, and/or a  
support.

20. (New) A conjugate comprising the compound of claim 10 conjugated to a probe, an FETL, a  
target, and/or a support.